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The Use of Decapsulated Cysts of the Brine Shrimp *Artemia* as Direct Food for Carp *Cyprinus carpio* L. Larvae

PAUL VANHAECKE,[1] LIEVEN DE VRIEZE, WIM TACKAERT AND PATRICK SORGELOOS[2]

Laboratory of Aquaculture & Artemia Reference Center, State University of Ghent, Rozier 44, B-9000 Ghent, Belgium

Abstract

Decapsulated *Artemia* cysts have been evaluated as a direct food source for larvae of the carp *Cyprinus carpio* L. All decapsulated cyst diets gave excellent survival of carp larvae during the first two weeks of culturing. Unlike traditionally brine-stored decapsulated cysts, dried *Artemia* embryos provided growth results comparable to those obtained with freshly hatched *Artemia* nauplii. Furthermore, except for *Artemia* embryos that lost their hatchability after long-term storage in air, several other inactivation treatments, simulating improper harvesting and processing of cysts, did not produce a significant decrease in the nutritional quality of the decapsulated cysts.

Using decapsulated cysts as a direct food source for carp larvae, instead of nauplii, the quantity of cysts needed can be reduced by about 25% to 35% after one and two weeks of culturing, respectively. Moreover, the use of decapsulated cysts advances the possible commercialization of poor-hatching and less expensive cyst products for culture of carp larvae.

Although freshly hatched *Artemia* nauplii are generally used as a nutritionally adequate and much more convenient alternative to natural zooplankton in intensive carp culture, their replacement by a more practical inert diet is still given a great deal of attention (Dabrowski et al. 1978, 1984; Bryant and Matty 1981; Kouril et al. 1981). *Artemia* cysts of high hatching quality are often expensive. The daily production of nauplii is labor intensive and requires additional facilities.

In order to overcome these drawbacks, the use of decapsulated *Artemia* cysts, cysts from which the nondigestible shell has been chemically removed, may offer an interesting alternative to live *Artemia* nauplii. Decapsulated cysts can be handled as an inert diet, do not leach and have a chemical composition comparable to the freshly hatched nauplii (Schauer et al. 1980; Seidel et al. 1980). In addition, their individual dry weight and energy content is on the average 30 to 40% higher than freshly hatched nauplii (Vanhaecke et al. 1983).

Decapsulated *Artemia* cysts have been reported as a direct food source for marine shrimp (Royan 1980; Bruggeman et al. 1980) and milkfish larvae (de los Santos et al. 1980). Recently, Verreth and Den Bieman (1987) and Verreth et al. (1987) reported on the quantitative requirements of the African catfish, *Clarias gariepinus*, for decapsulated cysts of *Artemia*. Although they obtained excellent results, comparative data using freshly hatched live nauplii were not given.

In this study the use of decapsulated cysts as a first food for carp larvae was quantified and compared to freshly hatched nauplii. Unlike freeswimming nauplii, decapsulated cysts float at the water surface when they are dry, and sink to the bottom when they become hydrated. Therefore, the first part of the study focused on methodology of culturing and feeding in order to optimize the availability of decapsulated cysts to the fish larvae. In a second experiment, the nutritional quality of decapsulated cysts was studied in relation to different inactivation methods which influence their hatchability.

[1] Present address: Ecolas, Noorderlaan 147, B-2030 Antwerpen, Belgium.

[2] Corresponding author.

Materials and Methods

Artemia cysts used in this study (in 1984) originated from Macau, Brazil (1982 harvest). Cyst incubation and preparation of freshly hatched nauplii were performed according to Vanhaecke and Sorgeloos (1983a). Cysts were decapsulated following procedures outlined in Bruggeman et al. (1980).

Decapsulated cysts were stored in a saturated NaCl brine. Prior to feeding, cysts were thoroughly washed with tapwater (1 min), blotted on a paper towel (1 min) and weighed to the nearest mg. The cysts were then resuspended in 100 ml of tapwater and stored in a refrigerator (12–14 C) for 24 h in cylindroconical tubes aerated from the bottom. Every day a fresh stock of cysts was prepared. The dry decapsulated cysts were prepared as follows: brine-stored cysts were thoroughly washed with tapwater, excess water was removed by squeezing cysts in a 100 μm nylon screen, cysts were distributed on a 100 μm screen in a layer <5 mm thick and dried at 35 C in a well ventilated room for 24 h.

Each 8 L experimental unit (aquaria unless specified otherwise) was supplied with aerated tapwater at a rate of 4 L/h and equipped with a 200 μm screened overflow tube. Three replicate units were used per treatment. Each test unit was stocked with 100 carp larvae two days after hatching. Feeding was started on the following day (day 1).

The feeding schedule, expressed in percent of live body weight/day was based on the data of Bryant and Matty (1980; Vanhaecke and Sorgeloos 1983b). Equal dry weight quantities of each food type were provided. The feed rations were adapted to the growth rate of the carp larvae as reported in Vanhaecke and Sorgeloos (1983b) and, unless specified otherwise, distributed over five feeding periods between 0900 and 1900. Dead carp larvae, unconsumed food and feces were removed daily. In each unit the amount of food distributed was adjusted for fish mortality. Dissolved oxygen levels were checked daily. The photoperiod was kept constant at 14 h light.

On day eight a subsample of 15 to 20 fish larvae was removed from each unit and weighed (Vanhaecke and Sorgeloos 1983b). On day 15 the wet weights of all remaining fish larvae were determined.

Experiment 1. Feeding Technique

In order to prevent or limit cyst sedimentation and to improve food availability for carp larvae, the following feeding treatments of decapsulated cysts were tested and compared to manual feeding of *Artemia* nauplii: 1) manual feeding of brine-stored decapsulated cyst; 2) continuous food distribution of brine-stored decapsulated cyst using a peristaltic pump; 3) continuous food distribution of brine-stored decapsulated cyst using an 8 L cylindroconical vessel provided with a continuous water flow (0.6 L/min), upwelling from the bottom, to keep the cysts in suspension; and 4) continuous food distribution of dried decapsulated cysts (sieved through a 220 μm screen and suspended in water) using a peristaltic pump. In order to prevent breaking and hatching of cysts during the feeding trials, decapsulated cysts were inactivated by UV irradiation (Sorgeloos 1979).

Experiment 2. Effect of Inactivation Method

Prior to drying, decapsulated cysts fed to carp larvae were subjected to the following inactivation treatments: UV irradiation; heating of hydrated cysts at 60 C; freezing of hydrated cysts (−20 C); slow dehydration (layer thickness of 1.5 cm, 30 C) and long term storage in air (cysts from the Macau 1979 harvest).

Both survival and wet-weight data were analyzed for statistical significance with a one-way analysis of variance (Model I; Sokal and Rohlf 1969). Survival data were normalized through an arcsin $\sqrt{\%}$ transformation. Duncan's Multiple Range test was used to identify individual treatment differences (Snedecor and Cochran 1967).

TABLE 1. *Survival and growth of carp larvae fed decapsulated* Artemia *cysts and nauplii using different feeding and culturing techniques.*

Diet	Experimental unit	Food distribution	Mean survival ± SD (%)	Mean individual weight ± SD (mg) 7 days	14 days
Decapsulated cysts	aquarium	manual (5×/day)	98.0 ± 1.0	8.2[c] ± 0.2	49.8[d] ± 2.4
Decapsulated cysts	aquarium	peristaltic pump (continuously)	98.7 ± 1.2	8.8[b,c] ± 0.7	60.6[c] ± 6.0
Dry decapsulated cysts (UV inactivated)	aquarium	manual (5×/day)	99.3 ± 0.6	10.1[b] ± 0.5	77.6[b] ± 5.1
Decapsulated cysts	cylindroconical vessel	peristaltic pump	98.3 ± 0.6	7.8[c] ± 1.0	50.1[d] ± 3.2
Nauplii	aquarium	manual (5×/day)	98.7 ± 0.6	14.3[a] ± 0.9	109.0[a] ± 7.9

[1] All results grouped by the same letter are not significantly different at the $P < 0.05$ level.

Results

The amount of dry weight naupliar biomass that could be produced from 1 g of cyst product (i.e., hatching output, Vanhaecke and Sorgeloos 1983a) averaged 445.6 mg. The individual dry weights of the decapsulated cysts and of the freshly hatched nauplii were 2.33 μg and 1.9 μg, respectively. The dry matter content of the decapsulated cysts and of the freshly hatched nauplii averaged 46.4 ± 1% and 19.8 ± 0.6%, respectively.

One hundred g of untreated cysts yielded an average of 73% dried decapsulated cysts (water content 3.9 ± 0.6%).

Experiment 1. Feeding Technique

Mean test temperature during this experiment (measured at 0900 and 1900) was 22.5 ± 0.7 C. It should be noted, however, that due to insufficient insulation, temperatures at night dropped to 19–20 C. Dissolved oxygen never dropped below 4.8 mg/L.

Feeding decapsulated cysts resulted in very high survival rates of carp larvae during the test period (Table 1). Larvae fed freshly hatched nauplii exhibited a significantly ($P < 0.05$) higher growth than those fed decapsulated cysts. Active feeding on cysts that had accumulated on the bottom was very limited. Floating (dry) decapsulated cysts, however, were actively taken from the surface from day 3 or 4 onwards. Among all decapsulated cyst diets, dry decapsulated cysts yielded the best growth rate. Continuous addition of cysts improved their availability and resulted in a significantly higher weight gain compared to manual feeding. The use of cylindroconical vessels with continuous water flow did not improve food availability.

Experiment 2. Effect of Inactivation Method

Mean test temperature during this experiment was 23.4 ± 0.7 C. Levels of dissolved oxygen were the same as in experiment 1.

The inactivation treatments of decapsulated cysts using UV irradiation and heating of hydrated cysts at 60 C resulted in total mortality of the embryos. Freezing and slow dehydration resulted in a decrease of the hatchability of decapsulated cysts from 77% to 20% and 35%, respectively. The cyst sample stored for five years in air, yielded only 30% viable cysts.

Survival of the carp larvae was not affected by the inactivation treatments used on the decapsulated cysts (Table 2). Weight gain of the carp larvae fed decapsulated cysts was significantly poorer than when freshly hatched nauplii were used after 1 week of culture. No significant differences were obtained between inactivated and viable embryos. Among the inactivation treatments, few significant differences were observed.

TABLE 2. *Survival and growth of carp larvae fed decapsulated* Artemia *cysts inactivated by different treatments.*

Inactivation method	Mean survival ± SD (%)	Mean individual weight (mg)	
		7 days	14 days
Freezing (−20 C; 48 h)	99.3 ± 0.6	$15.7^{b,c}$ ± 0.9	$127.5^{a,b,c}$ ± 4.7
Temperature shock (60 C)	99.7 ± 0.6	14.5^{c} ± 0.3	118.6^{c} ± 1.1
Slow dehydration (30 C; 72 h)	99.3 ± 0.6	16.4^{b} ± 1.3	$127.0^{a,b,c}$ ± 7.6
UV treatment (15 min)	99.3 ± 0.6	$15.2^{b,c}$ ± 0.4	$120.0^{b,c}$ ± 3.5
Long term storage in air (5 years)	99.7 ± 0.6	14.0^{c} ± 0.3	95.9^{d} ± 8.5
Viable embryos (no activation)	98.7 ± 1.2	$15.8^{b,c}$ ± 1.1	$129.5^{a,b}$ ± 2.9
Nauplii	99.7 ± 0.6	18.8^{a} ± 1.5	132.2^{a} ± 6.2

[1] All results grouped by the same letter are not significantly different at the $P < 0.05$ level.

Only long term storage in air and temperature shock treatments produced a significant decrease in carp growth at day 7 as compared to slow dehydration. After two weeks, individual weight differences between larvae fed nauplii, viable cysts, and cysts inactivated by freezing and slow dehydration were no longer significant. Inactivation resulting from temperature shock, UV treatment and long term storage in air were the only treatments producing significantly reduced growth of carp larvae after two weeks of culturing.

Discussion

Our results of growth and survival obtained with a diet of decapsulated *Artemia* cysts confirm the earlier findings of Anwand et al. (1976) and Grudniewski et al. (1978) that carp larvae readily accept an inert diet. However, when decapsulated cysts are stored in NaCl brine, i.e., the traditional storage method (Bruggeman et al. 1980), they cannot be used successfully because they sink too rapidly to be available to carp larvae under practical culturing conditions. The use of dry (floating) decapsulated cysts greatly improves the availability of cysts to carp larvae and ensures very good survival of carp larvae during the critical first week of culturing. The growth rate achieved after two weeks when using viable embryos (mean weight 129.5 mg) was comparable to that obtained with freshly hatched nauplii (mean weight 132.3 mg). The lower weight gain after one week on a decapsulated cyst diet was probably related to reduced consumption during the first 24 to 48 hours of culturing.

The results of this study show that, in addition to viable decapsulated cysts, inactivated or dead embryos are an acceptable food for carp larvae. This means that cysts with low hatchability due to improper harvesting or processing techniques, e.g., periodic hydration–dehydration (Rakowicz 1975), exposure to high temperatures (Voronov 1974) or slow dehydration (Clegg and Cavagnaro 1976), are no longer useless for aquaculture application. This might open new marketing opportunities for the large stocks (estimated at several 100 tons) of low-hatching cysts in the USA, USSR and PR China.

Long term exposure of cysts to air and ambient temperatures may have induced biochemical changes leading to a reduced nutritional value of the cysts, e.g., Jackson et al. (1990) reported that air storage of decapsulated cysts at 20 C for 15 weeks resulted in an important decrease in the amounts of the essential fatty acid 18:3w3. Another hypothesis for the apparently reduced performance of carp larvae fed decapsulated cysts exposed to air might be that the scrupulous sifting of that particular sample of decapsulated cysts (contrary to all other test samples, no aggregates of two or more cysts were present in the sample of old cysts) was responsible for the lower weight gain of carp larvae. Especially during the second week, carp larvae might have had to

TABLE 3. *Evaluation of cyst requirements for the production of 1 g of carp biomass.*

	Mean individual dry weight of carp larvae (g)		DW of food (g) provided per carp larva		Quantity of cysts (g) needed for production of 1 g carp biomass (% reduction when using decapsulated cysts)	
	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
Experiment 2						
Naupliar biomass	0.0188	0.1323	0.01383	0.09432	1.65	1.60
Viable dry decapsulated cysts	0.0158	0.1295	0.01383	0.09432	1.20 (27)	0.59 (38)

spend relatively more energy in feeding on the undersized food particles. Kouril et al. (1981) and Vanhaecke and Sorgeloos (1983b) reported that growth of carp larvae is directly correlated to the size of the zooplankton or *Artemia* nauplii being fed. The latter concluded that, as long as the size of the food particle does not interfere with the ingestion mechanism of the carp larvae, the use of large *Artemia* will be beneficial.

Taking into account that 1 g of cyst material yields 0.4456 g dry weight (DW) naupliar biomass and 0.730 g DW decapsulated cysts, the quantity of cysts needed per carp larva, using live nauplii and decapsulated cysts can be calculated by dividing the amount of DW of food (in g) provided per carp larva by 0.4456 and 0.730 respectively. Then, the Food Conversion Ratio or quantity of cysts (g) needed for the production of 1 g carp biomass (A) can be calculated using the following formula:

$$A = B/C$$

where

B = quantity of cysts (in g) needed to produce one carp larva
C = mean individual dry weight DW (in g) of carp larvae.

The use of decapsulated cysts instead of nauplii can result in an important reduction in the quantity of cyst product needed (Table 3). Because common carp larvae need live food for a minimum of one week (Coche and Bianchi 1979; Bryant and Matty 1981) approximately one quarter of the *Artemia* cyst product needed to produce nauplii can be saved. This amounts to a savings of over one third after two weeks of culture, when decapsulated cysts are used instead of nauplii.

A complete economic evaluation of the use of decapsulated cysts implies that the labor and costs involved in the decapsulation and drying of the cysts should be taken into account. However, labor and time spent in decapsulating and drying cysts is largely compensated because daily incubation, separation, harvest and storage of nauplii during feeding become unnecessary. Chemicals for decapsulation (NaOCl, NaOH, HCl and NaCl) of cysts are also inexpensive (approx. US $3.5 for the production of 1 kg of decapsulated cysts) in comparison to the cost of cysts (approx. US $25.0 per kg). The use of decapsulated cysts as food for larval carp is an attractive alternative to nauplii because an off-the-shelf product eliminates the risk of unforeseen shortage of live food.

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